Recommendations for the Invalidation of Test Results
(1/3/94)

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From: Director: Center for Biologics Evaluation and Research

Subject: Recommendations for the Invalidation of Test Results When Using Licensed Viral Marker Assays to Screen Donors

To: All Registered Blood and Plasma Establishments

Introduction

To reduce viral transmission by blood products, blood and blood components are tested for viral markers, including hepatitis B surface antigen, and antibodies to human immunodeficiency viruses type 1 and type 2, hepatitis C virus encoded antigen, human T-cell lymphotropic virus type I, hepatitis B virus core antigen and syphilis. Assay results are accepted as valid or rejected as invalid based on manufacturer's test kit product insert acceptance/rejection criteria. These criteria are based on the results obtained using control reagents included in the test kits (internal controls). The attached recommendations are intended to clarify the FDA position on the invalidation of test results when screening donor blood using licensed viral marker assays, including the use of external control reagents. External control reagents are defined as those control reagents which are not a component of the test kit and are either purchased commercially or developed by the blood establishment.

On May 28, 1992 and on September 25, 1992, the Blood Products Advisory Committee (BPAC) discussed, in open public meetings, circumstances for invalidating viral marker test results when screening donations with FDA licensed test kits. The discussions included general aspects of quality assurance related to viral marker testing, invalidation procedures based on licensed test kit product inserts, and procedures to invalidate test results based on criteria not included in the product insert. As a result of these discussions, FDA has developed recommendations for blood establishments desiring to supplement test kit product insert acceptance/rejection criteria. Accordingly, blood establishments may choose to develop procedures to include quality control rules that are consistent with, but may be more stringent than, test kit product insert rejection criteria. These procedures should be consistent with generally accepted quality control practices and should be part of a comprehensive quality assurance program *.

[*Footnote: Guideline for Quality Assurance in Blood Establishments 8 (Draft), June 17, 1993, Docket No. 91N-0450, Food and Drug Administration, HFM-12, 1401 Rockville Pike, Rockville, MD, 20852-1448]

Because blood safety relies significantly on the accuracy of viral marker testing, user-developed acceptance/rejection rules should be designed only with the objective of enhancing safety. Accordingly, rules developed by blood establishments may be used to invalidate nonreactive results, but must not be used to invalidate reactive results obtained from assay runs that satisfy the manufacturer's criteria for acceptance. If no error in a test procedure has been recognized, the reactive results remain as the initial test of record, and these specimens should be retested in duplicate as required by the test kit manufacturer's instructions. The duplicate retest is recommended to reduce the probability of obtaining a false negative result for a truly positive, but borderline reactive, specimen. An invalidated nonreactive result may be retested singly.

Implementation of reliable quality assurance and quality control procedures, including supplemental procedures that may involve external control reagents, should contribute to overall testing accuracy and blood safety.

/s/
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When Using Licensed Viral Marker Assays to Screen Donors

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These recommendations are intended for all blood establishments that wish to include in their Standard Operating Procedures for screening donor blood methods by which to invalidate test results, based on site-developed quality control procedures in addition to test kit package insert rejection criteria. Both reactive and nonreactive results should be invalidated when it has been determined that the assay has not been performed according to the test kit package insert requirements (e.g., improper procedure, compromised reagent, faulty equipment, etc.) or that the assay run fails to meet package insert defined acceptance criteria (e.g., controls out of specified range). If the blood establishment develops quality control rules supplemental to the test kit package insert instructions, they may be used to invalidate nonreactive test results. However, rules developed by blood establishments must not be used to invalidate reactive test results obtained with assays that meet the manufacturer's specifications. Specimens from collections

with reactive test results should be further assayed and reported as recommended in FDA Memoranda to Blood Establishments (1,2,3,4,5) regarding viral marker testing of donations.

I. Invalidation of Reactive and Nonreactive Test Results Using Package Insert Rejection Criteria.

A. All test results should be invalidated if the results for the test kit-supplied control reagents, i.e., internal control reagents, fail to meet package insert acceptance criteria. Thus, in this circumstance, both reactive and nonreactive results would be rejected and a subsequent assay would become the initial test of record.

B. All test results should be invalidated if it is determined that the assay was not performed in accordance with requirements stated in the test kit manufacturer's package insert. This includes correct performance of the test procedure, proper reagent quality (e.g., within-date, no evidence of contamination) and equipment that meets specifications. The determination of nonadherence to package insert requirements can be a result of an investigation into an unexplained discrepancy, a routine review of the assay data, or concerns raised by a blood establishment's supplementary quality control procedures. For example, an investigation of procedures may be performed in response to unexpected external control reagent values or failure to meet the blood establishments's supplementary statistical acceptance criteria for rate of reactivity.

II. Invalidation of Nonreactive Test Results Using Supplementary Acceptance/Rejection Criteria

The use of quality control procedures supplemental to the manufacturer's instructions may augment blood safety efforts. Such procedures may be capable of identifying unreliable test results and additionally may alert the firm that the risk of significant error may be increasing.

A. Initial Tests

1. Use of External Positive Controls to Invalidate Nonreactive Test Results

Rejection criteria developed by the firm for external control reagents may be used to invalidate only <u>nonreactive</u> test results in an assay run. Reactive test results may not be invalidated, and those reactive results remain as the initial tests of record. Reactive

specimens should then be tested in duplicate in the repeat test.

2. Use of Other Criteria to Reject Nonreactive Test Results

Rejection criteria based on donor population data, e.g., an unexpectedly increased reactive rate within a test run, may serve as a basis for invalidation of nonreactive test results, and the repeated assay, performed once on each donation, becomes the initial test of record for the nonreactive specimens. However, reactive specimens in the assay run may not be invalidated (unless the run fails package insert requirements as described in I.A. and I.B.), and those reactive results remain as the initial tests of record. Reactive specimens should then be tested in duplicate in the repeat test.

Should test run data indicate an unexpectedly increased reactive rate, the blood establishment may elect to perform the duplicate retests using the same procedure, but employing a different kit lot of the same manufacturer's product. This strategy takes into account minor lot-to-lot specificity variations which are inherent in these types of test kits.

B. Duplicate Repeat Testing

When an assay run is valid by test kit acceptance criteria and both of the repeated duplicate tests are nonreactive but additional supplemental quality control acceptance criteria, as in II.A.1. and 2. above, are not met, such duplicate test results may be invalidated. This donor should be retested again, in duplicate, and this second duplicate test then becomes the test of record, providing all other acceptance criteria have been met. However, if either of the original repeated duplicates is reactive, the donor must be classified as repeatedly reactive and no further repeat testing should be performed.

It must be emphasized that reactive results may only be invalidated when an assay run either fails to meet package insert acceptance criteria or the assay was not performed in accordance with the test kit package insert.

Accordingly, invalidation of reactive test results may not be based solely on either external control reagent performance or supplemental quality control rules, e.g., external control reagent values exceeding site established quality control acceptance limits, reactive rates exceeding site-defined upper limits, etc.

Whenever any test results are invalidated, documentation should include the basis for invalidation, and the details of an investigation, including records of supervisory oversight, the outcome of the investigation, and, if indicated, any corrective action taken. All of these actions should be taken prior to repeat testing of donor samples.

1. Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 23 April 1992, Center for Biologics Evaluation and Research, FDA.

2. Recommendations for the Management of Donors and Units that are Initially Reactive for Hepatitis B Surface Antigen (HBsAg), 2 December 1987, Center for Biologics Evaluation and Research, FDA.

3. Revised Recommendations for the Prevention of Human Immunodeficiency Virus (HIV) Transmission by Blood and Blood Products, 23 April 1992, Center for Biologics Evaluation and Research, FDA.

4. FDA Recommendations Concerning Testing for Antibody to Hepatitis B Core Antigen (Anti-HBc), 10 September 1992, Center for Biologics Evaluation and Research, FDA.

5. HTLV-I Antibody Testing, 29, September 1989, Center for Biologics Evaluation and Research, FDA.